Law Offices
FOLEY & LARDNER
3000 K Street, N.W., Suite 500
P.O. Box 25696
Washington, D.C. 20007-8696
(202) 672-5300

Assistant Commissioner for Patents
Washington, D. C. 20231

APPLICATION UNDER 37 C.F.R. 1.53(b)

Sir:

Transmitted herewith for filing is the patent application of:

INVENTOR(S): David W. LEUNG and Christopher K. TOMPKINS

TITLE: HUMAN PHOSPHATIDIC ACID PHOSPHATASE

In connection with this application, the following are enclosed:

_23_ Pages of Specification with Abstract

_12_ Claims

_13_ Sheets of Drawings

_XX_ Declaration, Power of Attorney to be filed under provisions of 37 C.F.R. 1.53(d)

____ Information Disclosure Statement/PTO-1449/

____ Certified Priority Application and Priority Claim

____ Statement of Small Entity Status

____ Other:

The fee has been calculated as shown below. (Small entity fees indicated in parentheses.)

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Kindly advise the undersigned of the period of time within which to file the oath or declaration of the inventors and TOTAL FEE.

Respectfully submitted,

John P. Isacson
Reg. No. 33,715

Date: April 17, 1997
Docket No.: 077319/0125

Attorney Docket No. 077319/0125

Inventors: **David W. Leung**
Christopher K. Tompkins

HUMAN PHOSPHATIDIC ACID PHOSPHATASE

Field of the Invention

This invention relates to human phosphatidic acid phosphatase. More particularly, this invention relates to three variants of human phosphatidic acid phosphatase namely PAP-α(1 and 2), PAP-β and PAP-γ and uses thereof. The invention encompasses biotechnology inventions, including biotechnology products and processes.

Background of the Invention

Phosphatidic acid phosphatase (PAP) (also referred to in the art as phosphatidate phosphohydrolase) is known to be an important enzyme for glycerolipid biosynthesis. In particular, PAP catalyzes the conversion of phosphatidic acid (PA) (also referred to in the art as phosphatidate) into diacylglycerol (DAG). DAG is an important branch point intermediate just downstream of PA in the pathways for biosynthesis of glycerophosphate-based phospholipids (Kent, Anal. Rev.Biochem. 64: 315-343, 1995).

In eukaryotic cells, PA, the precursor molecule for all glycerophospholipids, is converted either to CDP-diacylglycerol (CDP-DAG) by CDP-DAG synthase (CDS) or to DAG by phosphatidic acid phosphatase (PAP). In mammalian cells, CDP-DAG is the precursor to phosphatidylinositol (PI), phosphatidylglycerol (PG), and cardiolipin (CL); whereas diacylglycerol is the precursor to triacylglycerol (TG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) in all eukaryotic cells. Therefore, the partitioning of phosphatidic acid between

CDP-diacylglycerol and diacylglycerol is an important regulatory point in eukaryotic phospholipid metabolism (Shen *et al.,* J. Biol. Chem. 271: 789-795, 1996).

In addition to being an important enzyme for glycerolipid biosynthesis, PAP is also an important enzyme for signal transduction. PAP catalyses the dephosphorylation of PA to DAG. DAG is a well-studied lipid second messenger which is essential for the activation of protein kinase C (Kent, Anal. Rev.Biochem. 64: 315-343, 1995); whereas PA itself is also a lipid messenger implicated in various signaling pathways such as NADPH oxidase activation and calcium mobilization (English, Cell Signal. 8: 341-347, 1996). The regulation of PAP activity can therefore affect the balance of divergent signaling processes that the cell receives in terms of PA and DAG (Brindley *et al.,* Chem. Phys. Lipids 80: 45-57, 1996).

Various forms of PAP have been isolated in porcine (Kai *et al.,* J. Biol. Chem. 271: 18931-18938, 1996) and rat species (Brindley *et al.,* Chem. Phys. Lipids 80: 45-57, 1996). Furthermore, the putative amino acid sequence of murine PAP has been identified. Kai *et al., supra*. Prior to the instant invention, however, human PAP had not been identified or isolated.

Genes coding for PAP have been identified in *E. coli* (Dillon *et al,* J. Biol. Chem. 260: 12078-12083, 1985) and in mouse (Kai *et al.,* J. Biol. Chem. 271: 18931-18938, 1996). Furthermore, the following GenBank human cDNA clones are available: accession nos. H17855, N75714, and W70040. No uses were known, however, for these polynucleotide sequences.

Accordingly, there is a need for the identification and isolation of human PAP and for methods of using human

PAP, for example, for the dephosphorylation of a substrate.

Summary of the Invention

It is therefore an object of the present invention to provide a polynucleotide sequences encoding three or more variants of human PAP, namely PAP-α(1 and 2), PAP-β and PAP-γ.

It is a further object to provide the isolated protein of these three variants.

It is yet a further object to provide a biotechnology method for preparing these variants via recombinant methods.

It is a further object to provide a biotechnology method of using these variants or human PA in general to synthesize DAG.

In accomplishing these and other objects there is provided an isolated polynucleotide encoding human phosphatidic acid phosphatase wherein the polynucleotide encodes a protein comprising a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2, and (iii) the sequence at amino acid number 1 to amino acid number 276 in Figure 4.

There is further provided an isolated human phosphatidic acid phosphatase protein, wherein the protein comprises a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2, and (iii) the sequence at amino acid number 1 to amino acid number 276 in Figure 4.

There if further provided a method of preparing a human phosphatidic acid phosphatase-β protein comprising the steps of (i) transforming a host cell with an

expression vector comprising a polynucleotide encoding human phosphatidic acid phosphatase, (ii) culturing the transformed host cells which express the protein and (iii) isolating the protein.

There if further provided a method of dephosphorylating a substrate comprising contacting the substrate with an effective amount of isolated human phosphatidic acid phosphatase protein such that the protein catalyzes the dephosphorylation of the substrate. It is further provided that the substrate of this method is selected from the group consisting of phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate. It is further provided that this method occurs *in vitro*, and comprises a step of isolating the dephosphoryled substrate. Additionally, the method can occur *in vivo*, and is effected by the administration of human phosphatidic acid phosphatase to a mammal in need thereof.

Brief Description of the Drawings

Figure 1 shows the DNA sequence of the cDNA insert of the human PAP-α1 isolated herein and the corresponding amino acid sequence.

Figure 2 shows the DNA sequence of the cDNA insert of the human PAP-α2 isolated herein and the corresponding amino acid sequence.

Figure 3 shows the DNA sequence of the cDNA insert of the human PAP-β isolated herein and the corresponding amino acid sequence.

Figure 4 shows the DNA sequence of the cDNA insert of the human PAP-γ isolated herein and the corresponding amino acid sequence.

Figure 5 shows amino acid sequences alignment of the murine PAP coding sequence and the coding sequences for human PAP-α(1 and 2), PAP-β and PAP-γ.

Figure 6 shows the effect of IL-1β on PAP-β

expression in human endothelial ECV304 cells using Northern blot analysis.

Figure 7 depicts a thin layer chromatography analysis demonstrating the increase in PA dephosphorylation in cells transfected with either the PAP-α1 or PAP-α2 cDNA expression plasmids.

Figure 8 shows the differential expression of PAP-α mRNA in various tumor versus normal tissues.

Figure 9 is a schematic representation of glycerophospholipid biosynthesis involving the conversion of PA to either DAG or CDP-DAG. The synthesis of PA to DAG involves the PAP enzyme, while the synthesis of PA to CPD-DAG involves the CDS enzyme.

Detailed Description of Preferred Embodiments

This invention relates to isolated human phosphatidic acid phosphatase. More particularly, this invention relates to three variants of human phosphatidic acid phosphatase namely PAP-α(1 and 2), PAP-β and PAP-γ.

Examples of the uses for human PAP include the following. PAP is an important tool for enzymatic catalysis of several biologically significant proteins. As discussed above, PAP catalyzes the dephosphorylation of PA to DAG. DAG, in turn, is essential for the activation of protein kinase C (Kent, Anal. Rev.Biochem. 64: 315-343, 1995).

Moreover, PAP catalyzes the dephosphorylation of lysophosphatidic acid (LPA), ceramide 1-phosphate (C-1-P), and sphingosine 1-phosphate (S-1-P) (Brindley *et al.*, *Chem. Phys. Lipids* 80: 45-57, 1996). In the case of LPA, S-1-P, and C-1-P, the products of the PAP reaction are monoacylglycerol, sphingosine, and ceramide, respectively. PAP can control the balance of a wide spectrum of lipid mediators of cell activation and signal transduction by modulating the phosphorylated state of these lipids.

Additionally, the human PAP of the present invention are likely to define a new family of tumor suppressor genes that can be used as candidate genes for gene therapy for the treatment of certain tumors. The relationship of PAP and tumor suppression is evidenced in findings that PAP activity is lower in fibroblast cell lines transformed with either the *ras* or *fps* oncogene than in the parental rat1 cell line (Brindley *et al.*, *Chem. Phys. Lipids* 80: 45-57, 1996). Decrease in PAP activity in transformed cells correlates with a concomitant increase in PA concentration. Moreover, elevated PAP activity and lower level of PA has been observed in contact-inhibited fibroblasts relative to proliferating and transformed fibroblasts (Brindley *et al.*, *Chem. Phys. Lipids* 80: 45-57, 1996). Therefore, PAP plays a role in decreasing cell division and as such can provide a useful tool in treating cancer.

Additionally, PA, the substrate for the enzyme PAP, has been implicated in cytokine induced inflammatory responses (Bursten *et al.*, Circ. Shock 44: 14-29, 1994; Abraham *et al.*, J. Exp. Med. 181: 569-575, 1995; Rice *et al.*, Proc. Natl. Acad. Sci. USA 91: 3857-3861 1994; Leung *et al.*, Proc. Natl. Acad. Sci. USA 92: 4813-4817, 1995) and the modulation of numerous protein kinases involved in signal transduction (English *et al.*, Chem. Phys. Lipids 80: 117-132, 1996). Because of the possibility that activation of human PAP expression can counter-balance the inflammatory response from cytokine stimulation through degradation of excess amount of PA in cells, the genes encoding human PAP can be used in gene therapy for the treatment of inflammatory diseases.

Human PAP described herein can also be used in gene therapy for the treatment of obesity associated with diabetes. PAP activity is decreased in the livers and hearts of the grossly obese and insulin resistant JCR:LA corpulent rat compared to the control lean phenotype

(Brindley *et al.*, *Chem. Phys. Lipids* 80: 45-57, 1996). Human PAP described herein therefore can provide an important tool for the treatment of obesity associated with diabetes.

1. Human PAP

As used herein, "phosphatidic acid phosphatase" or "PAP" refers to a protein capable of catalyzing the dephosphorylation of PA to DAG. PAP also includes proteins capable of catalyzing the dephosphorylation of lysophosphatidic acid (LPA), ceramide 1-phosphate (C-1-P), and sphingosine 1-phosphate (S-1-P).

As used herein, "isolated" PAP denotes a degree of separation of the protein from other materials endogenous to the host organism. As used herein, "purified" denotes a higher degree of separation than isolated. A purified protein is sufficiently free of other materials endogenous to the host organism such that any remaining materials do not adversely affect the biological properties of the protein, for example, a purified protein is one sufficiently pure to be used in a pharmaceutical context.

As used herein, "human" PAP refers to PAP naturally occurring (or "native") in the human species, including natural variations due to allelic differences. The term "human PAP," however, is not limited to native human proteins, but also includes amino acid sequence variants of native human PAP that demonstrate PAP activity, as defined above.

Variants often exhibit the same qualitative biological activity as the naturally-occurring analogue, although variants also are selected in order to modify the characteristics of PAP protein. In a preferred embodiment, therefore, human PAP includes the amino acid sequences of Figures 1-4, being PAP-α1, PAP-α2, PAP-β and PAP-γ, respectively and variants thereof.

Amino acid sequence variants of the protein can be substitutional, insertional or deletion variants. Deletion variants lack one or more residues of the native protein which are not essential for biological activity. An example of a common deletion variant is a protein lacking transmembrane sequences. Another example is a protein lacking secretory signal sequences or signal sequences directing the protein to bind to a particular part of a cell.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and are designed to modulate one or more properties of the protein such as stability against proteolytic cleavage. Substitutions preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparigine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparigine; glutamate to aspartate; glycine to proline; histidine to asparigine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Of course, other amino acid substitutions can be undertaken.

Insertional variants contain fusion proteins such as those used to allow rapid purification of the protein and also can include hybrid proteins containing sequences from other proteins and polypeptides which are protein homologues.

Variants of human PAP also include fragments,

analogs, derivatives, muteins and mimetics of the natural PAP protein that retain the ability to cause the beneficial results described above. Fragments of the human PAP protein refer to portions of the amino acid sequence of the PAP polypeptide that also retain this ability.

Variants can be generated directly from the human PAP protein itself by chemical modification by proteolytic enzyme digestion, or by combinations thereof. Additionally, methods of synthesizing polypeptides directly from amino acid residues also exist.

Non-peptide compounds that mimic the binding and function of the human PAP protein ("mimetics") can be produced by the approach outlined in Saragovi *et al.*, *Science* 253: 792-95 (1991). Mimetics are peptide-containing molecules which mimic elements of protein secondary structure. See, for example, Johnson *et al.*,"Peptide Turn Mimetics" in BIOTECHNOLOGY AND PHARMACY, Pezzuto *et al.*, Eds., (Chapman and Hall, New York, 1993).

The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions For the purposes of the present invention, appropriate mimetics can be considered to be the equivalent of the human PAP protein itself.

More typically, at least in the case of gene therapy, variants are created by recombinant techniques employing genomic or cDNA cloning methods. Site-specific and region-directed mutagenesis techniques can be employed. See CURRENT PROTOCOLS IN MOLECULAR BIOLOGY vol. 1, ch. 8 (Ausubel *et al.* eds., J. Wiley & Sons 1989 & Supp. 1990-93); PROTEIN ENGINEERING (Oxender & Fox eds., A. Liss, Inc. 1987). In addition, linker-scanning and PCR-mediated techniques can be employed for

mutagenesis. See PCR TECHNOLOGY (Erlich ed., Stockton Press 1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra*. Protein sequencing, structure and modeling approaches for use with any of the above techniques are disclosed in PROTEIN ENGINEERING, *loc. cit.* and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vols. 1 & 2, *supra*.

2. Polynucleotides Encoding Human PAP

The present invention further includes isolated polynucleotides encoding human phosphatidic acid phosphatase. As used herein, an "isolated" polynucleotide denotes a degree of separation of the polynucleotide from its naturally occurring environment, *e.g.*, from its native intact genome. In a preferred embodiment, the isolated polynucleotides correspond to those shown in Figure 1 at nucleotide number 342 to nucleotide number 1193; Figure 2 at nucleotide number 342 to nucleotide number 1196; Figure 3 at amino acid number 1 to amino acid number 311; and Figure 4 at nucleotide number 4 to nucleotide number 833.

The invention furthermore relates to a polynucleotide whose sequence is degenerate with respect to the sequences mentioned above in accordance with the nature of the genetic code. Degeneracy is often referred to as codon/anticodon wobble, and is discussed in Watson *et al.*, MOLECULAR BIOLOGY OF THE GENE (4th ed. 1987) at 437-43.

The present invention further includes bases, nucleosides, nucleotides, oligonucleotides derived from the isolated polynucleotides of the present invention. The term "derived" when used in the context of the present invention connotes a degree of similarity that is sufficient to indicate the original polynucleotide from which hybrid forms, or portions thereof, were obtained. Also within the scope of the invention are so-

called "polyamide" or "peptide" nucleic acids ("PNAs") derived from the polynucleotides of the present invention. PNAs are constructed by replacing the (deoxy)ribose phosphate backbone of a subject polynucleotide with an achiral polyamide backbone or the like. See Nielsen *et al.*, *Science* 254: 1497-54 (1991).

The above polynucleotides and derivations thereof can be used as important tools in recombinant DNA and other protocols involving nucleic acid hybridization techniques. More specifically, oligonucleotides and nucleic acids derived from the isolated polynucleotides shown in Figures 1-4 can be used as hybridization probes, capable of recognizing and specifically binding to complementary nucleic acid sequences, providing thereby a means of detecting, identifying, locating and measuring complementary nucleic acid sequences in a biological sample.

Biological samples include, among a great many others, blood or blood serum, lymph, ascites fluid, urine, microorganism or tissue culture medium, cell extracts, or the like, derived from a biological source, or a solution containing chemically synthesized protein, or an extract or solution prepared from such fluid from a biological source.

An oligonucleotide containing a modified nucleotide of the invention can be used as a primer to initiate nucleic acid synthesis at locations in a DNA or RNA molecule comprising the sequence complementary to the oligonucleotide sequence. The synthesized nucleic acid strand would have incorporated, at its 5' terminus, the oligonucleotide primer bearing the invention and would, therefore, be detectable by exploitation of the characteristics of the detectable label. Two such primers, specific for different nucleotide sequences on complementary strands of dsDNA, can be used in the polymerase chain reaction (PCR) to synthesize and amplify

the amount of a nucleotide sequence. The detectable label present on the primers will facilitate the identification of desired PCR products. PCR, combined with techniques for preparing complementary DNA (cDNA) can be used to amplify various RNAs, with oligonucleotide primers again serving both to provide points for initiation of synthesis in the cDNA duplex flanking the desired sequence and to identify the desired product. Primers labeled with the invention may also be utilized for enzymatic nucleic acid sequencing by the dideoxy chain-termination technique.

The invention can be applied to measure or quantitate the amount of DNA present in a sample. For instance, the concentration of nucleic acid can be measured by comparing detectable labels incorporated into the unknown nucleic acid with the concentration of detectable labels incorporated into known amounts of nucleic acid.

Such a comparative assessment can be done using biotin where the respective concentrations are determined by an enzyme-linked assay utilizing the streptavidin-alkaline phosphatase conjugate and a substrate yielding a soluble chromogenic or chemiluminescent signal.

3. Recombinant Production of Human PAP

In a further embodiment human PAP is expressed via recombinant methods known to those of skill in the art. The polynucleotides of the present invention can be expressed in any number of different recombinant DNA expression systems to generate large amounts of protein, which can then be purified and used for the various applications of human PAP described above. Included within the present invention are proteins having native glycosylation sequences, and deglycosylated or unglycosylated proteins prepared by the methods described below.

Recombinant technology for producing desired proteins is known by ordinarily skilled artisans and includes providing a coding sequence for a desired protein, and operably linking the coding sequence to polynucleotide sequences capable of effecting its expression.

With regard to one aspect of the invention, it often is desirable to produce human PAP as a fusion protein, freed from upstream, downstream or intermediate sequences, or as a protein linked to leader sequences, effecting secretion of human PAP into cell culture medium.

A typical expression system will also contain control sequences necessary for transcription and translation of a message. Known control sequences include constitutive or inducible promoter systems, translational initiation signals (in eucaryotic expression), polyadenylation translation termination sites, and transcription terminating sequences. Expression vectors containing controls which permit operably linking of desired coding sequences to required control systems are known by the skilled artisan. Such vectors can be found which are operable in a variety of hosts.

Human PAP of the present invention may be produced in procaryotic cells using appropriate controls, such as *trp* or *lac* promoters, or in eucaryotic host cells, capable of effecting post-translational processing that permits proteins to assume desired three-dimensional conformation. Eucaryotic control systems and expression vectors are known; including *leu* and glycolytic promoters useful in yeast, the viral SV40 and adenovirus and CMV promoters in mammalian cells, and the baculovirus system which is operable in insect cells. Plant vectors with suitable promoters, such as the *nos* promoter are also available.

Standard laboratory manuals (*e.g.*, Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Second Edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989) present standard techniques and methodologies for expressing polynucleotides encoding a desired protein, culturing appropriate cells, providing suitable expression conditions, and recovering a resulting protein from culture.

In preparing the inventive human PAP, a suitable polynucleotide encoding human PAP, constructed utilizing any of the foregoing techniques is operable linked to an expression vector which is then transformed into a compatible host. Host cells are cultured using conditions appropriate for growth. Expression of the desired human PAP is preferably induced after some predetermined growth level has occurred. Human PAP production is monitored and the desired protein isolated from culture either from a supernatant, or by first lysing host cells with an appropriate agent, or by other methods known to the skilled artisan.

In another preferred embodiment, a polynucleotide encoding human PAP is ligated into a mammalian expression vector. A preferred mammalian expression vector is the plasmid "pCE2." The plasmid pCE2 is derived from pREP7b (Leung, et al., Proc. Natl. Acad. Sci. USA, 92: 4813-4817, 1995) with the RSV promoter region replaced by the CMV enhancer and the elongation factor-1α (EF-1α) promoter and intron. The CMV enhancer of the pCE2 vector is constructed from a 380 bp Xba I-Sph I fragment produced by PCR from pCEP4 (Invitrogen, San Diego, CA) using the primers 5'-GGCTCTAGAT ATTAATAGTA ATCAATTAC-3' and 5'-CCTCACGCAT GCACCATGGT AATAGC-3'. The EF-1α promoter and intron (Uetsuki, et al., J. Biol. Chem., 264: 5791-5798, 1989) are constructed from a 1200 bp Sph I-Asp718 I fragment produced by PCR from human genomic DNA using the primers 5'-GGTGCATGCG TGAGGCTCCG GTGC-3'

and 5'-GTAGTTTTCA CGGTACCTGA AATGGAAG-3'. These 2 fragments are ligated into a Xba I/Asp718 I digested vector derived from pREP7b to generate pCE2.

In another preferred embodiment of the present invention, pCE2 containing a polynucleotide expressing human PAP is used to transform a host cell which then expresses the protein. Preferred host cells include the human embryonic kidney cell line 293-EBNA (Invitrogen, San Diego, CA), endothelial ECV304 cells, and epithelial A549 cells.

4. Dephosphorylation of Substrate

In another embodiment, the present invention includes a method of dephosphorylating a substrate by contacting the substrate with an effective amount of isolated human PAP. An "effective amount" of human PAP is an amount which will dephosphorylate a detectable amount of substrate. Such an amount can be determined empirically based on variables well known to those of skill in the art, such as reaction time and temperature.

In one embodiment, the substrate includes phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate. In another embodiment, the isolated human PAP includes PAP-α(1 and 2), PAP-β and PAP-γ and variants thereof.

In a further embodiment, the dephosphorylation of substrate occurs *in vitro*, by contacting a substrate with recombinantly produced human PAP expressed by the methods described above. The dephosphorylated substrate is then isolated by standard isolation and purification methods, including for example, thin layer chromatography or high pressure liquid chromatography.

In another embodiment, the dephosphorylation of substrate occurs *in vivo* via the administration of human PAP to a mammal, preferably a human. "Administration" means delivery of human PAP protein to a mammal by

methods known to those of skill in the art including, but not limited to: orally, for example in the form of pills, tablets, lacquer tablets, coated tablets, granules, hard gelatin capsules, soft gelatin capsules, solutions, syrups, emulsions, suspensions or aerosol mixtures; rectally, for example in the form of suppositories; parenterally, for example in the form of injection solutions or infusion solutions, microcapsules or rods; percutaneously, for example in the form of ointments or tinctures; transdermally; intravascularly, intracavitarily; intramuscularly; subcutaneously; and nasally, for example in the form of nasal sprays or inhalants.

The administration of human PAP protein includes the administration of the protein combined in a mixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g. human serum albumin, are described for example in Remington's *Pharmaceutical Sciences* by E.W. Martin, which is hereby incorporated by reference. Such compositions will contain an effective amount of protein hereof together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host.

Such compositions should be stable for appropriate periods of time, preferably are acceptable for administration to humans and preferably are readily manufacturable. Although pharmaceutical solution formulations are provided in liquid form appropriate for immediate use, formulations may also be provided in frozen or in lyophilized form. In the former case, the composition must be thawed prior to use. The latter form is often used to enhance the stability of the medicinal agent contained in the composition under a wide variety of storage conditions. Such lyophilized preparations are reconstituted prior to use by the addition of suitable

pharmaceutically acceptable diluents, such as sterile water or sterile physiological saline solution.

Additionally, administration is meant to include delivery of human PAP protein to a mammal by means of gene therapy techniques, *i.e.*, by the delivery of polynucleotides encoding human PAP to PAP-deficient cells, whereby human PAP is then expressed in the cell. Gene therapy techniques are known to those of skill in the art. For example, listing of present-day vectors suitable for use in gene therapy of the present invention is set forth in Hodgson, *Bio/Technology* 13: 222 (1995). *See also*, Culver *et al.*, *Science*, 256:1550-62 (1992).

Additionally, liposome-mediated gene transfer is another suitable method for the introduction of a recombinant vector containing a polynucleotide encoding human PAP into a PAP-deficient cell. *See* Caplen *et al.*, *Nature Med.* 1:39-46 (1995) and Zhu *et al.*, *Science* 261:209-211 (1993).

Additionally, viral vector-mediated gene transfer is also a suitable method for the introduction of a recombinant vector containing the gene encoding human PAP into a PAP-deficient cell. Examples of appropriate viral vectors are adenovirus vectors. Detailed discussions of the use of adenoviral vectors for gene therapy can be found in Berkner, *Biotechniques* 6:616-629 (1988), Trapnell, *Advanced Drug Delivery Rev.* 12:185-199 (1993).

The following examples merely illustrate the invention and, as such, are not to be considered as limiting the invention set forth in the claims.

Example 1
Cloning and Expression of Human PAP-α, PAP-β and PAP-γ

Homology search of the Genbank database (Boguski, et al., Science 265:1993-1994, 1994) of expressed sequence tag (dbEST) using the murine PAP protein sequence (Kai *et al.*, J. Biol. Chem. 271: 18931-18938,

1996) as probe identified several short stretches of human cDNA sequences with homology to the murine PAP protein sequence. These cDNA sequences of interest were derived from single-run partial sequencing of random human cDNA cloning projects carried out mainly by I.M.A.G.E. Consortium [LLNL] cDNA clones program. Based on the partial DNA sequences available in the GenBank database, the human cDNA clones that are homologous to the murine PAP protein sequence can be grouped into three classes, suggesting the presence of at least three different human PAP variants, designated as PAP-α, PAP-β, and PAP-γ here. For instance, a potential human PAP-α clone (GenBank #H17855) identified contains sequence homologous to aa 272-283 and the 3'-untranslated region of murine PAP; a potential human PAP-β clone (GenBank #W70040) identified contains sequence similarities corresponding to aa 175-251 of murine PAP; and a potential human PAP-γ clone (GenBank #N75714) identified contains sequences similarities corresponding to aa 18-142 of murine PAP. These cDNA clones were purchased (Genome Systems, St. Louis, MO) for further analysis. DNA sequence determination of the entire cDNA inserts of these clones showed clone H17855 contained sequences that are homologous to the N- and C-terminal sequences of murine PAP with a gap of about 150 bp that led to a frame shift in reading frame. This clone is most likely a spuriously spliced form of PAP-α clone. Clone W70040 was found to be a full-length PAP-β clone, and clone N75714 was found to be a partial PAP-γ clone with an open reading frame homologous to the region from aa18 to the C-terminus of murine PAP.

To assemble a full-length functional PAP-α clone, synthetic oligonucleotides o_papa1F, 5'-ggcatggtAC CATGTTTGAC AAGACGCGGC-3', based on the N-terminal region of PAP-α and o_papa1R, 5'-CATATGTAGT ATTCAATGTA ACC-3', based on a region downstream of a Pst I site

complementary to the coding strand of PAP-α were used to amplify the N-terminal coding region of PAP-α from a human lung cDNA library (Life Technologies, Inc., Gaithersburg, MD). The 450 bp Acc65 I - Pst I fragment generated was inserted into a Acc65 I / Pst I vector from pBluescript(II)SK(-) (Stratagene, San Diego, CA) for further analysis. DNA sequence analysis of the subclones obtained revealed at least two different classes of clones with sequences that diverged at the putative exon of interest, suggesting the presence of two alternatively spliced forms of PAP-α. These two alternatively spliced forms of PAP-α are designated as PAP-α1 and PAP-α2 here. Each of the individual 450 bp Acc65 I - Pst I fragment generated by PCR was combined with the 810 bp Pst I - Not I fragment derived from clone H17855 for ligation into a Acc65 I / Not I mammalian expression vector derived from pCE2 for the generation of expression plasmids for PAP-α1 and PAP-α2. The plasmid pCE2 was derived from pREP7b (Leung, et al., Proc. Natl. Acad. Sci. USA, 92: 4813-4817, 1995) with the RSV promoter region replaced by the CMV enhancer and the elongation factor-1α (EF-1α) promoter and intron. The CMV enhancer of the pCE2 vector was constructed from a 380 bp Xba I-Sph I fragment produced by PCR from pCEP4 (Invitrogen, San Diego, CA) using the primers 5'-GGCTCTAGAT ATTAATAGTA ATCAATTAC-3' and 5'-CCTCACGCAT GCACCATGGT AATAGC-3'. The EF-1α promoter and intron (Uetsuki, et al., J. Biol. Chem., 264: 5791-5798, 1989) was constructed from a 1200 bp Sph I-Asp718 I fragment produced by PCR from human genomic DNA using the primers 5'-GGTGCATGCG TGAGGCTCCG GTGC-3' and 5'-GTAGTTTTCA CGGTACCTGA AATGGAAG-3'. These 2 fragments were ligated into a Xba I/Asp718 I digested vector derived from pREP7b to generate pCE2.

The DNA sequence determined from clone N75714 was used as a probe to search for clones with overlapping sequences in the GenBank database. Clone Z43618 was

found to contain an additional 5'-sequence with a potential ATG initiation codon. To assemble a full-length PAP-γ clone, synthetic oligonucleotides o_papg1F, 5'-tgatggctag cATGCAGAGA AGATGGGTCT TCGTGCTGCT CGACGTG-3', based on the N-terminal region of PAP-γ and o_papg1R, 5'-AGTGCGGGAT CCCATAAGTG GTTG-3', based on a region complementary to the coding strand of PAP-γ just downstream of its stop codon were used to generate the full-length coding region of PAP-γ by PCR using the clone N75714 as template. The 820 bp Nhe I - BamH I fragment obtained was then ligated into a Nhe I / BamH I mammalian expression vector derived from pCE2.

Figures 1, 2, 3 and 4 show the translated DNA sequences of the putative human cDNA clones for PAP-α1, α2, β and γ, respectively. The designated ATG initiation site for translation of each cDNA clone fulfills the requirement for an adequate initiation site according to Kozak (Kozak, Critical Rev. Biochem. Mol. Biol. 27:385-402, 1992).

The amino acid sequence of each open reading frame (Figures 1, 2, 3 and 4) was used as the query sequence to search for homologous sequences in protein databases. Search of the Genbank database from the National Center for Biotechnology Information (NCBI) using the blastp program showed that these proteins are most homologous to the murine PAP sequence (Kai *et al.*, J. Biol. Chem. 271: 18931-18938, 1996), and a rat endoplasmic reticulum resident transmembrane protein of unknown function, *Dri 42*, whose expression is up-regulated during epithelial differentiation (Barila *et al.*, J. Biol. Chem. 271: 29928-29936, 1996).

Example 2
Activation of PAP-β Transcription by IL1-β

It is possible that activation of PAP-β expression can counter-balance the inflammatory response from IL-1β

stimulation through degradation of the excess amount of PA in cells. To determine whether IL1-β, an inflammatory cytokine, would activate the transcription of PAP mRNAs, Northern analysis of PAP-β mRNA levels (Fig. 6) was performed in human endothelial ECV304 cells at various times after IL-1β stimulation. Figure 6 shows that PAP-β mRNA expression was induced after incubation of ECV304 cells with IL-1β after at least 6 hours, suggesting that PAP-β is a late-response gene to IL-1β stimulation. This indicates that human PAP may act to reduce IL-1β induced inflammation by degrading excess PA in cells.

Example 3
PAP-α1 and PAP-α2 Dephosphorylation of PA to DAG

The expression of PAP-α1 and PAP-α2 cDNA was found to increase PA dephosphorylation in mammalian cells. The expression plasmids for PAP-α1, PAP-α2 and the control vector were transiently transfected into 293-EBNA (EB293) cells (Invitrogen, San Diego, CA) using the lipofectant DOTAP (Boehringer Mannheim, Indianapolis, IN). PAP activities were followed by TLC analysis based on the conversion of [C^{14}]PA (DuPont NEN, Boston, MA) to [C^{14}]DAG using membrane fractions isolated from the various cell extracts. Figure 7 shows membrane fractions derived from cells transfected with either the PAP-α1 (lanes 6 and 7) or PAP-α2 (lanes 8 and 9) produced more [C^{14}]DAG those from untransfected cells (lanes 2 and 3) or from cells transfected with the control pCE2 vector (lanes 4 and 5). In this particular chromatography system, DAG can be resolved into two bands, possibly due to heterogeneity in the acyl-chains. It appears that PAP-α1 and PAP-α2 preferentially dephosphorylate different species of PA as evidenced by the change in relative intensity of the two DAG bands (lanes 6 to 9).

Example 4
Differential Expression of PAP-α mRNA in Selected Tumor Versus Normal Tissues

The possibility that PAP-α expression can degrade the excess amount of PA in cells suggests that PAP-α may be down-regulated in tumor cells when compared to normal cells, as tumor cells tend to be more inflammatory due to a possibly higher level of PA when compared to normal or resting cells. To test this hypothesis, Northern analysis using PAP-α(1 and 2) cDNA probe was performed on RNA blots derived from various matching pairs of tumor and normal tissues (Invitrogen, Carlsbad, CA). Figure 8 shows the expression levels of PAP-α mRNA are substantially higher in five out of eight of the normal tissues examined; namely, colon, rectal, breast, fallopian tube, and ovarian tissues when compared to the corresponding tumor tissues.

<u>What Is Claimed Is:</u>

1. An isolated polynucleotide encoding human phosphatidic acid phosphatase wherein said polynucleotide encodes a protein comprising a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2, and (iii) the sequence at amino acid number 1 to amino acid number 276 in Figure 4.

2. An isolated human phosphatidic acid phosphatase protein, wherein said protein comprises a polypeptide sequence selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2, and (iii) the sequence at amino acid number 1 to amino acid number 276 in Figure 4.

3. A method of preparing a human phosphatidic acid phosphatase-β protein comprising the steps of (i) transforming a host cell with an expression vector comprising a polynucleotide encoding human phosphatidic acid phosphatase, (ii) culturing said transformed host cells which express said protein and (iii) isolating said protein.

4. The method of claim 3, wherein said polynucleotide encoding human phosphatidic acid is selected from the group consisting of (i) the sequence at amino acid number 1 to amino acid number 284 in Figure 1, (ii) the sequence at amino acid number 1 to amino acid number 285 in Figure 2, (iii) the sequence at amino acid number 1 to amino acid number 311 in Figure 3, and (iv) the sequence at amino acid number 1 to amino acid number 276 in Figure 4.

5. A method of dephosphorylating a substrate comprising contacting said substrate with an effective amount of isolated human phosphatidic acid phosphatase protein such that said protein catalyzes the dephosphorylation of said substrate.

6. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 284 in Figure 1.

7. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 285 in Figure 2.

8. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 311 in Figure 3.

9. The method of claim 5, wherein said protein comprises the polypeptide sequence at amino acid number 1 to amino acid number 276 in Figure 4.

10. The method of claim 5, wherein said substrate is selected from the group consisting of phosphatidic acid, lysophosphatidic acid, ceramide 1-phosphate, and sphingosine 1-phosphate.

11. The method of claim 5, wherein said contacting is effected *in vitro*, and further comprises the step of isolating said dephosphoryled substrate.

12. The method of claim 5, wherein said contacting step occurs *in vivo* and is effected by the administration of said human phosphatidic acid phosphatase to a mammal in need thereof.

Abstract

This invention relates to a biotechnology invention concerning human phosphatidic acid phosphatase. More particularly, this invention relates to three variants of human phosphatidic acid phosphatase namely PAP-α(1 and 2), PAP-β and PAP-γ and uses thereof.

Docket No. 077319/0125

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

HUMAN PHOSPHATIDIC ACID PHOSPHATASE

the specification of which is attached hereto unless the following box is checked:

☒ was filed on *April 17, 1997* as United States Application Number or PCT International Application Number __________ and was amended on __________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

Address all correspondence to FOLEY & LARDNER, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696. Address telephone communications to *John P. Isacson* at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor	Signature of First or Sole Inventor	Date
David W. LEUNG		
Residence Address	**Country of Citizenship**	
Mercer Island, Washington	*USA*	
Post Office Address		
7625 E. Mercer Way, Mercer Island, Washington 98040		

Full Name of Second Inventor	Signature of Second Inventor	Date
Christopher K. TOMPKINS		
Residence Address	**Country of Citizenship**	
Bothell, Washington	*USA*	
Post Office Address		
17660 86th Avenue, N.E., Bothell, Washington 98011		

Figure 1. Translated sequence of human PAP-α1 cDNA.

```
CCTGTGGGAGAGAGCGCCGGGATCCGGACGGGGTAGCAACCGGGGCAGGCCGTGCCGGCTGA    62
GGAGGTCCTGAGGCTACAGAGCTGCCGCGGCTGGCACACGAGCGCCTCGGCACTAACCGA    122
GTGTTCGCGGGGGCTGTGAGGGGAGGGCCCCGGGCGCCATTGCTGGCGGTGGGAGCGCCG    182
CCCGGTCTCAGCCCGCCCTCGGCTGCTCTCCTCCTCCGGCTGGGAGGGGCCGTATCTCGG    242
GGCCGTCGCCAGCCCCGGCCCGGGCTCGATAATCAAGGGCCTCGGCCGTCGTCCCGCACC    302
TCATTCCATCGCCCTTGCCGGGCAGCCCGGGCAGAGACC ATG TTT GAC AAG ACG     356
                                        Met Phe Asp Lys Thr
                                                          5

CGG CTG CCG TAC GTG GCC CTC GAT GTG CTC TGC GTG TTG CTG GCT      401
Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys Val Leu Leu Ala
                 10                  15                  20
GGA TTG CCT TTT GCA ATT CTT ACT TCA AGG CAT ACC CCC TTC CAA      446
Gly Leu Pro Phe Ala Ile Leu Thr Ser Arg His Thr Pro Phe Gln
                 25                  30                  35
CGA GGA GTA TTC TGT AAT GAT GAG TCC ATC AAG TAC CCT TAC AAA      491
Arg Gly Val Phe Cys Asn Asp Glu Ser Ile Lys Tyr Pro Tyr Lys
                 40                  45                  50
GAA GAC ACC ATA CCT TAT GCG TTA TTA GGT GGA ATA ATC ATT CCA      536
Glu Asp Thr Ile Pro Tyr Ala Leu Leu Gly Gly Ile Ile Ile Pro
                 55                  60                  65
TTC AGT ATT ATC GTT ATT ATT CTT GGA GAA ACC CTG TCT GTT TAC      581
Phe Ser Ile Ile Val Ile Ile Leu Gly Glu Thr Leu Ser Val Tyr
                 70                  75                  80
TGT AAC CTT TTG CAC TCA AAT TCC TTT ATC AGG AAT AAC TAC ATA      626
Cys Asn Leu Leu His Ser Asn Ser Phe Ile Arg Asn Asn Tyr Ile
                 85                  90                  95
GCC ACT ATT TAC AAA GCC ATT GGA ACC TTT TTA TTT GGT GCA GCT      671
Ala Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala Ala
                100                 105                 110
GCT AGT CAG TCC CTG ACT GAC ATT GCC AAG TAT TCA ATA GGC AGA      716
Ala Ser Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly Arg
                115                 120                 125
CTG CGG CCT CAC TTC TTG GAT GTT TGT GAT CCA GAT TGG TCA AAA      761
Leu Arg Pro His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser Lys
                130                                     140
ATC AAC TGC AGC GAT GGT TAC ATT GAA TAC TAC ATA TGT CGA GGG      806
Ile Asn Cys Ser Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg Gly
                145                 150                 155
AAT GCA GAA AGA GTT AAG GAA GGC AGG TTG TCC TTC TAT TCA GGC      851
Asn Ala Glu Arg Val Lys Glu Gly Arg Leu Ser Phe Tyr Ser Gly
                160                 165                 170
CAC TCT TCG TTT TCC ATG TAC TGC ATG CTG TTT GTG GCA CTT TAT      896
His Ser Ser Phe Ser Met Tyr Cys Met Leu Phe Val Ala Leu Tyr
                175                 180                 185
CTT CAA GCC AGG ATG AAG GGA GAC TGG GCA AGA CTC TTA CGC CCC      941
Leu Gln Ala Arg Met Lys Gly Asp Trp Ala Arg Leu Leu Arg Pro
                190                 195                 200
ACA CTG CAA TTT GGT CTT GTT GCC GTA TCC ATT TAT GTG GGC CTT      986
Thr Leu Gln Phe Gly Leu Val Ala Val Ser Ile Tyr Val Gly Leu
                205                 210                 215
TCT CGA GTT TCT GAT TAT AAA CAC CAC TGG AGC GAT GTG TTG ACT      1031
Ser Arg Val Ser Asp Tyr Lys His His Trp Ser Asp Val Leu Thr
                220                 225                 230
GGA CTC ATT CAG GGA GCT CTG GTT GCA ATA TTA GTT GCT GTA TAT      1076
Gly Leu Ile Gln Gly Ala Leu Val Ala Ile Leu Val Ala Val Tyr
                235                 240                 245
GTA TCG GAT TTC TTC AAA GAA AGA ACT TCT TTT AAA GAA AGA AAA      1121
Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe Lys Glu Arg Lys
                250                 255                 260
```

Continuation of Figure 1.

```
GAG GAG GAC TCT CAT ACA ACT CTG CAT GAA ACA CCA ACA ACT GGG     1166
Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro Thr Thr Gly
                265                 270                 275
AAT CAC TAT CCG AGC AAT CAC CAG CCT TGA AAG GCAGCAGGGTGCCCAG    1215
Asn His Tyr Pro Ser Asn His Gln Pro ***
                280
GTGAAGCTGGCCTGTTTTCTAAAGGAAAATGATTGCCACAAGGCAAGAGGATGCATCTTT    1275
CTTCCTGGTGTACAAGCCTTTAAAGACTTCTGCTGCTGATATGCCTCTTGGATGCACACT    1335
TTGTGTGTACATAGTTACCTTTAACTCAGTGGTTATCTAATAGCTCTAAACTCATTAAAA    1395
AAACTCCAAGCCTTCCACCAAAACAGTGCCCCACCTGTATACATTTTTATTAAAAAAATG    1455
TAATGCTTATGTATAAACATGTATGTAATATGCTTTCTATGAATGATGTTTGATTTAAAT    1515
ATAATACATATTAAAATGTATGGGAGAACCAAAAAAAAAAAAAAAAAA                1563
```

Figure 2. Translated sequence of human PAP-α2 cDNA

CCTGTGGGAGAGAGCGCCGGGATCCGGACGGGGTAGCAACCGGGGCAGGCCGTGCCGGCTGA 62
GGAGGTCCTGAGGCTACAGAGCTGCCGCGGCTGGCACACGAGCGCCTCGGCACTAACCGA 122
GTGTTCGCGGGGGCTGTGAGGGGAGGGCCCCGGGCGCCATTGCTGGCGGTGGAGCGCCG 182
CCCGGTCTCAGCCCGCCCTCGGCTGCTCTCCTCCTCCGGCTGGGAGGGGCCGTATCTCGG 242
GGCCGTCGCCAGCCCCGGCCCGGGCTCGATAATCAAGGGCCTCGGCCGTCGTCCCGCACC 302
TCATTCCATCGCCCTTGCCGGGCAGCCCGGGCAGAGACC ATG TTT GAC AAG ACG 356
Met Phe Asp Lys Thr
5

CGG CTG CCG TAC GTG GCC CTC GAT GTG CTC TGC GTG TTG CTG GCT 401
Arg Leu Pro Tyr Val Ala Leu Asp Val Leu Cys Val Leu Leu Ala
10 15 20

TCC ATG CCT ATG GCT GTT CTA AAA TTG GGC CAA ATA TAT CCA TTT 446
Ser Met Pro Met Ala Val Leu Lys Leu Gly Gln Ile Tyr Pro Phe
25 30 35

CAG AGA GGC TTT TTC TGT AAA GAC AAC AGC ATC AAC TAT CCG TAC 491
Gln Arg Gly Phe Phe Cys Lys Asp Asn Ser Ile Asn Tyr Pro Tyr
40 45 50

CAT GAC AGT ACC GCC GCA TCC ACT GTC CTC ATC CTA GTG GGG GTT 536
His Asp Ser Thr Ala Ala Ser Thr Val Leu Ile Leu Val Gly Val
55 60 65

GGC TTG CCC GTT TCC TCT ATT ATT CTT GGA GAA ACC CTG TCT GTT 581
Gly Leu Pro Val Ser Ser Ile Ile Leu Gly Glu Thr Leu Ser Val
70 75 80

TAC TGT AAC CTT TTG CAC TCA AAT TCC TTT ATC AGT AAT AAC TAC 626
Tyr Cys Asn Leu Leu His Ser Asn Ser Phe Ile Ser Asn Asn Tyr
85 90 95

ATA GCC ACT ATT TAC AAA GCC ATT GGA ACC TTT TTA TTT GGT GCA 671
Ile Ala Thr Ile Tyr Lys Ala Ile Gly Thr Phe Leu Phe Gly Ala
100 105 110

GCT GCT AGT CAG TCC CTG ACT GAC ATT GCC AAG TAT TCA ATA GGC 716
Ala Ala Ser Gln Ser Leu Thr Asp Ile Ala Lys Tyr Ser Ile Gly
115 120 125

AGA CTG CGG CCT CAC TTC TTG GAT GTT TGT GAT CCA GAT TGG TCA 761
Arg Leu Arg Pro His Phe Leu Asp Val Cys Asp Pro Asp Trp Ser
130 135 140

AAA ATC AAC TGC AGC GAT GGT TAC ATT GAA TAC TAC ATA TGT CGA 806
Lys Ile Asn Cys Ser Asp Gly Tyr Ile Glu Tyr Tyr Ile Cys Arg
145 150 155

GGG AAT GCA GAA AGA GTT AAG GAA GGC AGG TTG TCC TTC TAT TCA 851
Gly Asn Ala Glu Arg Val Lys Glu Gly Arg Leu Ser Phe Tyr Ser
160 165 170

GGC CAC TCT TCG TTT TCC ATG TAC TGC ATG CTG TTT GTG GCA CTT 896
Gly His Ser Ser Phe Ser Met Tyr Cys Met Leu Phe Val Ala Leu
175 180 185

TAT CTT CAA GCC AGG ATG AAG GGA GAC TGG GCA AGA CTC TTA CGC 941
Tyr Leu Gln Ala Arg Met Lys Gly Asp Trp Ala Arg Leu Leu Arg
190 195 200

CCC ACA CTG CAA TTT GGT CTT GTT GCC GTA TCC ATT TAT GTG GGC 986
Pro Thr Leu Gln Phe Gly Leu Val Ala Val Ser Ile Tyr Val Gly
205 210 215

CTT TCT CGA GTT TCT GAT TAT AAA CAC CAC TGG AGC GAT GTG TTG 1031
Leu Ser Arg Val Ser Asp Tyr Lys His His Trp Ser Asp Val Leu
220 225 230

ACT GGA CTC ATT CAG GGA GCT CTG GTT GCA ATA TTA GTT GCT GTA 1076
Thr Gly Leu Ile Gln Gly Ala Leu Val Ala Ile Leu Val Ala Val
235 240 245

TAT GTA TCG GAT TTC TTC AAA GAA AGA ACT TCT TTT AAA GAA AGA 1121
Tyr Val Ser Asp Phe Phe Lys Glu Arg Thr Ser Phe Lys Glu Arg
250 260

Continuation of Figure 2

```
AAA GAG GAG GAC TCT CAT ACA ACT CTG CAT GAA ACA CCA ACA ACT      1166
Lys Glu Glu Asp Ser His Thr Thr Leu His Glu Thr Pro Thr Thr
                265                 270                 275
GGG AAT CAC TAT CCG AGC AAT CAC CAG CCT TGA AAGGCAGCAGGGTGCC     1215
Gly Asn His Tyr Pro Ser Asn His Gln Pro ***
                280                 285
CAGGTGAAGCTGGCCTGTTTCTAAAGGAAAATGATTGCCACAAGGCAAGAGGATGCATC      1275
TTTCTTCCTGGTGTACAAGCCTTTAAAGACTTCTGCTGCTGATATGCCTCTTGGATGCAC     1335
ACTTTGTGTGTACATAGTTACCTTTAACTCAGTGGTTATCTAATAGCTCTAAACTCATTA     1395
AAAAAACTCCAAGCCTTCCACCAAAACAGTGCCCCACCTGTATACATTTTTATTAAAAAA     1455
ATGTAATGCTTATGTATAAACATGTATGTAATATGCTTTCTATGAATGATGTTTGATTTA     1515
AATATAATACATATTAAAATGTATGGGAGAACCAAAAAAAAAAAAAAAAAA              1566
```

Figure 3. Translated sequence of PAP-β cDNA

```
GGCGCAGCTCTGCAAAAGTTTCTGCTCGGGATCTGGCTCTCTTCCCCTTGGACTTTAGAACG   62
ATTTAGGGTTGACAGAGGAAAGCAGAGGCGCGCAGGAGGAGCAGAAAACACCACCTTCTG    122
CAGTTGGAGGCAGGCAGCCCCGGCTGCACTCTAGCCGCCGCGCCCGGAGCCGGGGCCGAC    182
CCGCCACTATCCGCAGCAGCCTCGGCCAGGAGGCGACCCGGGCGCCTGGGTGTGTGGCTG    242
CTGTTGCGGGACGTCTTCGCGGGGCGGGAGGCTCGCGCCGCAGCCAGCGCC ATG CAA     299
                                                    Met Gln

AAC TAC AAG TAC GAC AAA GCG ATC GTC CCG GAG AGC AAG AAC GGC     344
Asn Tyr Lys Tyr Asp Lys Ala Ile Val Pro Glu Ser Lys Asn Gly
         5                  10                  15
GGC AGC CCG GCG CTC AAC AAC AAC CCG AGG AGG AGC GGC AGC AAG     389
Gly Ser Pro Ala Leu Asn Asn Asn Pro Arg Arg Ser Gly Ser Lys
        20                  25                  30
CGG GTG CTG CTC ATC TGC CTC GAC CTC TTC TGC CTC TTC ATG GCG     434
Arg Val Leu Leu Ile Cys Leu Asp Leu Phe Cys Leu Phe Met Ala
        35                  40                  45
GGC CTC CCC TTC CTC ATC ATC GAG ACA AGC ACC ATC AAG CCT TAC     479
Gly Leu Pro Phe Leu Ile Ile Glu Thr Ser Thr Ile Lys Pro Tyr
        50                  55                  60
CAC CGA GGG TTT TAC TGC AAT GAT GAG AGC ATC AAG TAC CCA CTG     524
His Arg Gly Phe Tyr Cys Asn Asp Glu Ser Ile Lys Tyr Pro Leu
        65                  70                  75
AAA ACT GGT GAG ACA ATA AAT GAC GCT GTG CTC TGT GCC GTG GGG     569
Lys Thr Gly Glu Thr Ile Asn Asp Ala Val Leu Cys Ala Val Gly
        80                  85                  90
ATC GTC ATT GCC ATC CTC GCG ATC ATC ACG GGG GAA TTC TAC CGG     614
Ile Val Ile Ala Ile Leu Ala Ile Ile Thr Gly Glu Phe Tyr Arg
        95                 100                 105
ATC TAT TAC CTG AAG AAG TCG CGG TCG ACG ATT CAG AAC CCC TAC     659
Ile Tyr Tyr Leu Lys Lys Ser Arg Ser Thr Ile Gln Asn Pro Tyr
       110                 115                 120
GTG GCA GCA CTC TAT AAG CAA GTG GGC TGC TTC CTC TTT GGC TGT     704
Val Ala Ala Leu Tyr Lys Gln Val Gly Cys Phe Leu Phe Gly Cys
       125                 130                 135
GCC ATC AGC CAG TCT TTC ACA GAC ATT GCC AAA GTG TCC ATA GGG     749
Ala Ile Ser Gln Ser Phe Thr Asp Ile Ala Lys Val Ser Ile Gly
       140                 135                 150
CGC CTG CGT CCT CAC TTC TTG AGT GTC TGC AAC CCT GAT TTC AGC     794
Arg Leu Arg Pro His Phe Leu Ser Val Cys Asn Pro Asp Phe Ser
       155                 160                 165
CAG ATC AAC TGC TCT GAA GGC TAC ATT CAG AAC TAC AGA TGC AGA     839
Gln Ile Asn Cys Ser Glu Gly Tyr Ile Gln Asn Tyr Arg Cys Arg
       170                                     180
GGT GAT GAC AGC AAA GTC CAG GAA GCC AGG AAG TCC TTC TTC TCT     884
Gly Asp Asp Ser Lys Val Gln Glu Ala Arg Lys Ser Phe Phe Ser
       185                 190                 195
GGC CAT GCC TCC TTC TCC ATG TAC ACT ATG CTG TAT TTG GTG CTA     929
Gly His Ala Ser Phe Ser Met Tyr Thr Met Leu Tyr Leu Val Leu
       200                 205                 210
TAC CTG CAG GCC CGC TTC ACT TGG CGA GGA GCC CGC CTG CTC CGG     974
Tyr Leu Gln Ala Arg Phe Thr Trp Arg Gly Ala Arg Leu Leu Arg
       215                 220                 225
CCC CTC CTG CAG TTC ACC TTG ATC ATG ATG GCC TTC TAC ACG GGA    1019
Pro Leu Leu Gln Phe Thr Leu Ile Met Met Ala Phe Tyr Thr Gly
       230                 235                 240
CTG TCT CGC GTA TCA GAC CAC AAG CAC CAT CCC AGT GAT GTT CTG    1064
Leu Ser Arg Val Ser Asp His Lys His His Pro Ser Asp Val Leu
       245                 250                 255
GCA GGA TTT GCT CAA GGA GCC CTG GTG GCC TGC TGC ATA GTT TTC    1109
Ala Gly Phe Ala Gln Gly Ala Leu Val Ala Cys Cys Ile Val Phe
```

Continuation of Figure 3

```
        260                     265                     270
TTC GTG TCT GAC CTC TTC AAG ACT AAG ACG ACG CTC TCC CTG CCT        1154
Phe Val Ser Asp Leu Phe Lys Thr Lys Thr Thr Leu Ser Leu Pro
        275                     280                     285
GCC CCT GCT ATC CGG AAG GAA ATC CTT TCA CCT GTG GAC ATT ATT        1199
Ala Pro Ala Ile Arg Lys Glu Ile Leu Ser Pro Val Asp Ile Ile
        290                     295                     300
GAC AGG AAC AAT CAC CAC AAC ATG ATG TAG GTGCCACCCACCTCCTGAGC       1249
Asp Arg Asn Asn His His Asn Met Met ***
        305                     310
TGTTTTTGTAAAATGACTGCTGACAGCAAGTTCTTGCTGCTCTCCAATCTCATCAGACAG       1309
TAGAATGTAGGGAAAAACTTTTGCCCGACTGATTTTTAAAAAAAAAAAAAAA               1362
```

Figure 4.   Translated sequence of human PAP-γ cDNA

```
ACC ATG CAG CGG AGG TGG GTC TTC GTG CTG CTC GAC GTG CTG TGC       47
    Met Gln Arg Arg Trp Val Phe Val Leu Leu Asp Val Leu Cys
                     5                  10
TTA CTG GTC GCC TCC CTG CCC TTC GCT ATC CTG ACG CTG GTG AAC       92
Leu Leu Val Ala Ser Leu Pro Phe Ala Ile Leu Thr Leu Val Asn
 15                 20                  25
GCC CCG TAC AAG CGA GGA TTT TAC TGC GGG GAT GAC TCC ATC CGG      137
Ala Pro Tyr Lys Arg Gly Phe Tyr Cys Gly Asp Asp Ser Ile Arg
 30                 35                  40
TAC CCC TAC CGT CCA GAT ACC ATC ACC CAC GGG CTC ATG GCT GGG      182
Tyr Pro Tyr Arg Pro Asp Thr Ile Thr His Gly Leu Met Ala Gly
 45                 50                  55
GTC ACC ATC ACG GCC ACC GTC ATC CTT GTC TCG GCC GGG GAA GCC      227
Val Thr Ile Thr Ala Thr Val Ile Leu Val Ser Ala Gly Glu Ala
 60                 65                  70
TAC CTG GTG TAC ACA GAC CGG CTC TAT TCT CGC TCG GAC TTC AAC      272
Tyr Leu Val Tyr Thr Asp Arg Leu Tyr Ser Arg Ser Asp Phe Asn
 75                 80                  85
AAC TAC GTG GCT GCT GTA TAC AAG GTG CTG GGG ACC TTC CTG TTT      317
Asn Tyr Val Ala Ala Val Tyr Lys Val Leu Gly Thr Phe Leu Phe
 90                 95                 100
GGG GCT GCC GTG AGC CAG TCT CTG ACA GAC CTG GCC AAG TAC ATG      362
Gly Ala Ala Val Ser Gln Ser Leu Thr Asp Leu Ala Lys Tyr Met
105                110                 115
ATT GGG CGT CTG AAG CCC AAC TTC CTA GCC GTC TGC GAC CCC GAC      407
Ile Gly Arg Leu Lys Pro Asn Phe Leu Ala Val Cys Asp Pro Asp
120                125                 130
TGG AGC CGG GTC AAC TGC TCG GTC TAT GTG CAG CTG GAG AAG GTG      452
Trp Ser Arg Val Asn Cys Ser Val Tyr Val Gln Leu Glu Lys Val
135                140                 145
TGC AGG GGA AAC CCT GCT GAT GTC ACC GAG GCC AGG TTG TCT TTC      497
Cys Arg Gly Asn Pro Ala Asp Val Thr Glu Ala Arg Leu Ser Phe
150                155                 160
TAC TCG GGA CAC TCT TCC TTT GGG ATG TAC TGC ATG GTG TTC TTG      542
Tyr Ser Gly His Ser Ser Phe Gly Met Tyr Cys Met Val Phe Leu
165                170                 175
GCG CTG TAT GTG CAG GCA CGA CTC TGT TGG AAG TGG GCA CGG CTG      587
Ala Leu Tyr Val Gln Ala Arg Leu Cys Trp Lys Trp Ala Arg Leu
180                185                 190
CTG CGA CCC ACA GTC CAG TTC TTC CTG GTG GCC TTT GCC CTC TAC      632
Leu Arg Pro Thr Val Gln Phe Phe Leu Val Ala Phe Ala Leu Tyr
195                200                 205
GTG GGC TAC ACC CGC GTG TCT GAT TAC AAA CAC CAC TGG AGC GAT      677
Val Gly Tyr Thr Arg Val Ser Asp Tyr Lys His His Trp Ser Asp
210                215                 220
GTC CTT GTT GGC CTC CTG CAG GGG GCA CTG GTG GCT GCC CTC ACT      722
Val Leu Val Gly Leu Leu Gln Gly Ala Leu Val Ala Ala Leu Thr
225                230                 235
GTC TGC TAC ATC TCA GAC TTC TTC AAA GCC CGA CCC CCA CAG CAC      767
Val Cys Tyr Ile Ser Asp Phe Phe Lys Ala Arg Pro Pro Gln His
240                245                 250
TGT CTG AAG GAG GAG GAG CTG GAA CGG AAG CCC AGC CTG TCA CTG      812
Cys Leu Lys Glu Glu Glu Leu Glu Arg Lys Pro Ser Leu Ser Leu
255                260                 265
ACG TTG ACC CTG GGG CGA GGC TGA CCACAACCACTTATGGGATACCCGCACT     864
Thr Leu Thr Leu Gly Arg Gly ***
270                275
CTTCTTCCTGAGGCCGGACCCCGCCCAGGCAGGGAGCTGCTGTGAGTCCAGCTGATGCCC     924
ACCCAGGTGGTCCCTCCAGCCTGGTTAGGCACTGAGGGTTCTGGACGGCTCCAGGAACC      984
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Continuation of Figure 4

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CTGGGCTGATGGGAGCAGTGAGCGGTTCCGCTGCCCCCTGCCCTGCACTGGACCAGGAGT   1044
CTGGAGATGCCTGGGTAGCCCTCAGCATTTGGAGGGGAACCTGTTCCCGTCGGTCCCCAA   1104
ATATCCCCTTCTTTTTATGGGGTTAAGGAAGGGACCGAGAGATCAGATAGTTGCTGTTTT   1164
GTAAAATGTAATGTATATGTGGTTTTTAGTAAAATAGGGCACCTGTTTCACAAAAAAAAA   1224
AAAAAAAAAA                                                     1234
```

Figure 5. Amino acid sequences alignment of murine PAP with the three human isoforms of PAP.

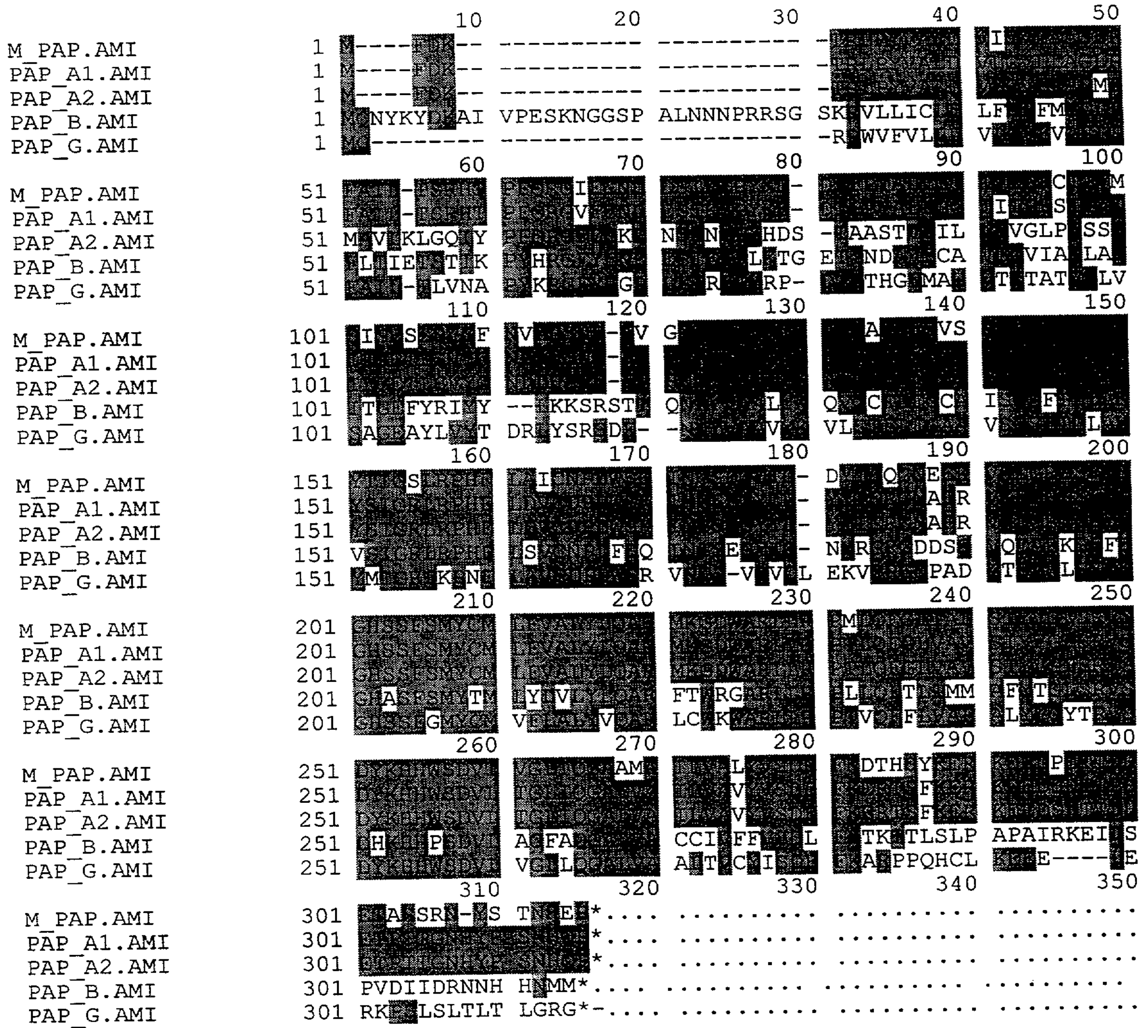


Figure 6.

Expression of PAP-β induced with Il-1β in ECV304 Cells

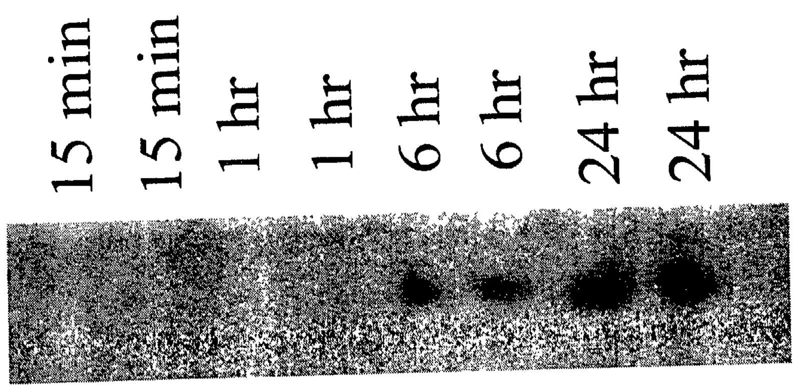
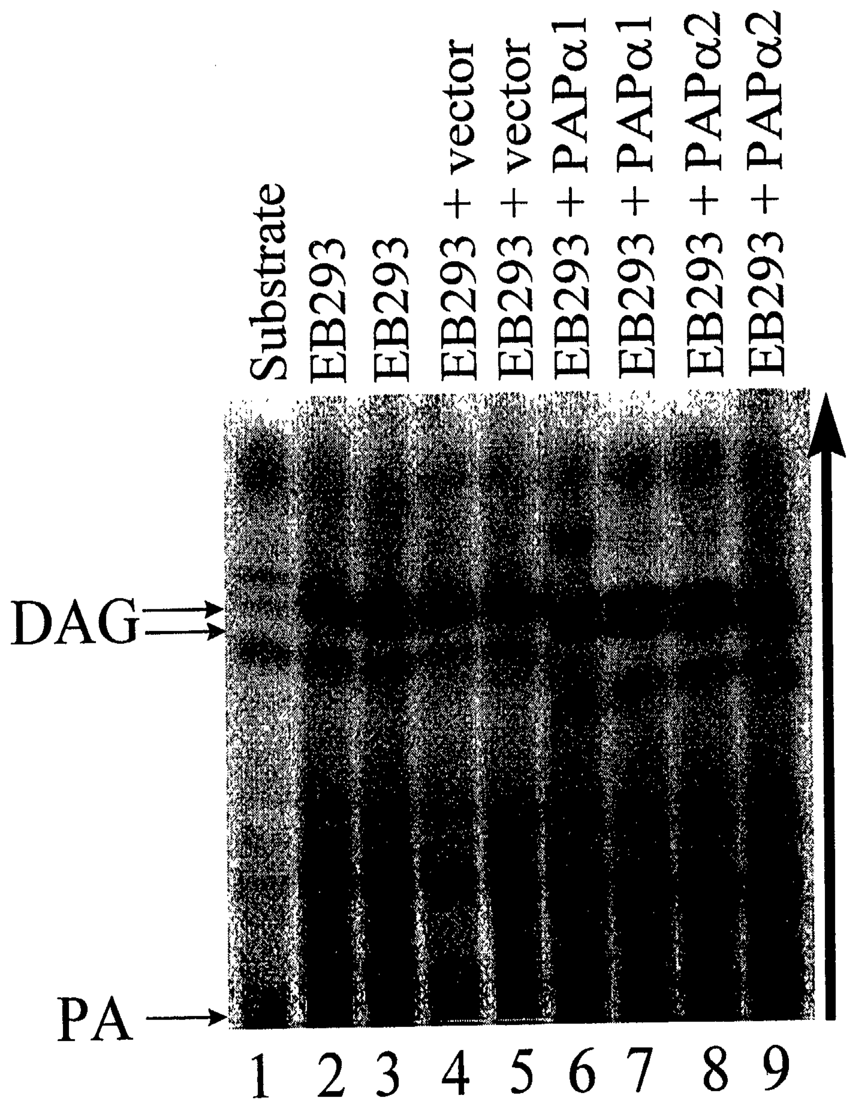


Figure 7


Substrate
EB293
EB293
EB293 + vector
EB293 + vector
EB293 + PAPα1
EB293 + PAPα1
EB293 + PAPα2
EB293 + PAPα2
DAG
PA
1 2 3 4 5 6 7 8 9

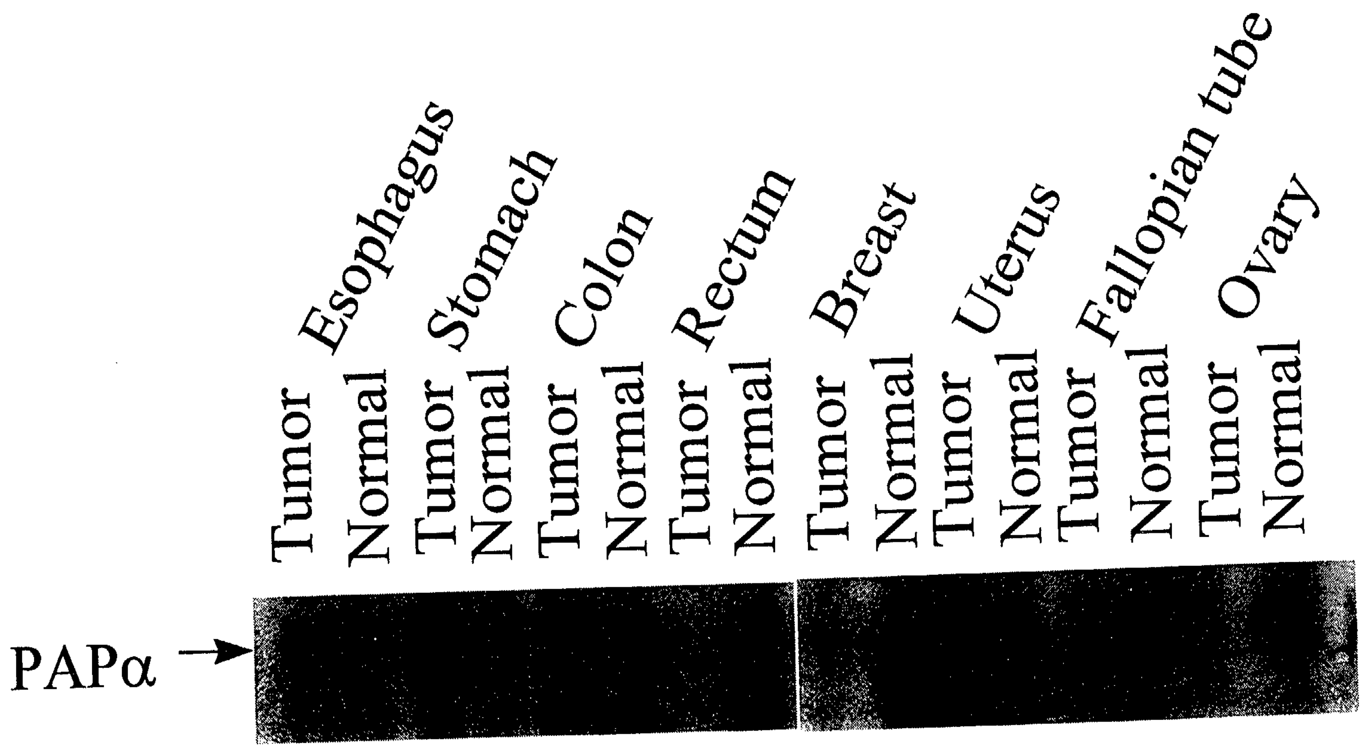
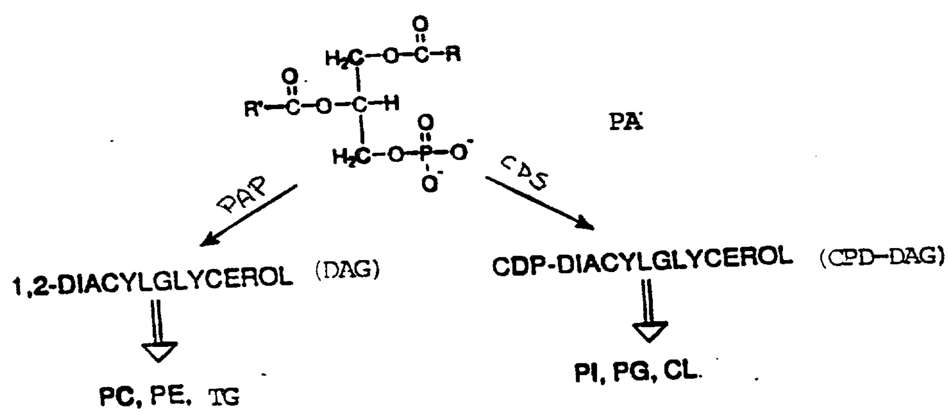


Fig. 8 Northern Analysis of PAP-α mRNA expression in tumor vs normal tissues

FIGURE 9


PA
PAP
CDS
1,2-DIACYLGLYCEROL (DAG)
CDP-DIACYLGLYCEROL (CPD-DAG)
PC, PE, TG
PI, PG, CL.